

We claim:

- Sub B1
- Sub C1
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1. A positionally addressable array comprising a plurality of different substances, selected from the group consisting of proteins, molecules comprising functional domains of said proteins, whole cells, and protein-containing cellular material, on a solid support, with each different substance being at a different position on the solid support, wherein the plurality of different substances consists of at least 100 different substances per  $\text{cm}^2$ .
  2. The array of claim 1 wherein the plurality of different substances consists of between 100 and 1,000 different substances per  $\text{cm}^2$ .
  3. The array of claim 1 wherein the plurality of different substances consists of between 1,000 and 10,000 different substances per  $\text{cm}^2$ .
  4. The array of claim 1 wherein the plurality of different substances consists of between 10,000 and 100,000 different substances per  $\text{cm}^2$ .
  5. The array of claim 1 wherein the plurality of different substances consists of between 100,000 and 1,000,000 different substances per  $\text{cm}^2$ .
  6. The array of claim 1 wherein the plurality of different substances consists of between 1,000,000 and 10,000,000 different substances per  $\text{cm}^2$ .
  7. The array of claim 1 wherein the plurality of different substances consists of between 10,000,000 and 25,000,000 different substances per  $\text{cm}^2$ .
  8. The array of claim 1 wherein the plurality of different substances consists of at least 25,000,000 different substances per  $\text{cm}^2$ .
  9. The array of claim 1 wherein the plurality of different substances consists of at least 10,000,000,000 different substances per  $\text{cm}^2$ .
  10. The array of claim 1 wherein the plurality of different substances consists of at least 10,000,000,000,000 different substances per  $\text{cm}^2$ .
  11. The array of claim 1 wherein the solid support is a glass slide.
  12. The array of claim 1 wherein each different substance is present in a different well on the surface of the solid support.
  13. The array of claim 12 wherein each different substance in a different well is bound to the surface of the solid support.
  14. The array of claim 12 wherein each different substance in a different well is not bound to the surface of the solid support.
  15. The array of claim 12 wherein each different substance in a different well is in solution.

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16. The array of claim 12 wherein each well contains reagents for assaying biological activity of a protein or molecule.
17. A positionally addressable array comprising a plurality of different proteins, or molecules comprising functional domains of said proteins, on a solid support, with each different protein or molecule being at a different position on the solid support, wherein the plurality of proteins or molecules consists of at least 50% of all expressed proteins with the same type of biological activity in the genome of an organism.
18. The array of claim 17 wherein the plurality of proteins or molecules consists of at least 75% of all expressed proteins with the same type of biological activity in the genome of an organism.
19. The array of claim 17 wherein the plurality of proteins or molecules consists of at least 90% of all expressed proteins with the same type of biological activity in the genome of an organism.
20. The array of claim 17 wherein the organism is selected from the group consisting of bacteria, yeast, insects, and mammals.
21. The array of claim 17 wherein the expressed proteins with a biological activity of interest are selected from the group consisting of kinases, phosphatases, proteases, glycosidases, acetylases, other group transferring enzymes, nucleic acid binding proteins, hormone binding proteins, and DNA binding proteins.
22. A positionally addressable array comprising a plurality of different substances selected from the group consisting of proteins, molecules comprising functional domains of said proteins, whole cells, and protein-containing cellular material, on a solid support, with each different substance being at a different position on the solid support, wherein the solid support is selected from the group consisting of ceramics, amorphous silicon carbide, castable oxides, polyimides, polymethylmethacrylates, polystyrenes and silicone elastomers.
23. The array of claim 22 wherein the solid support is silicone elastomer.
24. The array of claim 23 wherein the solid support is polydimethylsiloxane.
25. A positionally addressable array comprising a plurality of different substances, selected from the group consisting of proteins, molecules comprising functional domains of said proteins, whole cells, and protein-containing cellular material, on a solid support, with each different substance being at a different position on the solid support, wherein the plurality of different substances are attached to the solid support via a 3-glycidooxypropyltrimethoxysilane linker.

26. An array comprising a plurality of wells on the surface of a solid support wherein the density of the wells is at least 100 wells/cm<sup>2</sup>.
27. The array of claim 26 wherein the density of the wells is between 100 and 1,000 wells/cm<sup>2</sup>.
- 5 28. The array of claim 26 wherein the density of the wells is between 1,000 and 10,000 wells/cm<sup>2</sup>.
29. The array of claim 26 wherein the density of the wells is between 10,000 and 100,000 wells/cm<sup>2</sup>.
30. The array of claim 26 wherein the density of the wells is between 100,000 and 1,000,000 wells/cm<sup>2</sup>.
- 10 31. The array of claim 26 wherein the density of the wells is between 1,000,000 and 10,000,000 wells/cm<sup>2</sup>.
32. The array of claim 26 wherein the density of the wells is between 10,000,000 and 25,000,000 wells/cm<sup>2</sup>.
- 15 33. The array of claim 26 wherein the density of the wells is at least 25,000,000 wells/cm<sup>2</sup>.
34. The array of claim 26 wherein the density of the wells is at least 10,000,000,000 wells/cm<sup>2</sup>.
35. The array of claim 26 wherein the density of the wells is at least 10,000,000,000,000 wells/cm<sup>2</sup>.
- 20 36. The array of claim 26 wherein a plurality of different substances, selected from the group consisting of proteins, molecules comprising functional domains of said proteins, whole cells, and protein-containing cellular material, is present in the wells, with each different substance being present in a different well.
- 25 37. The array of claim 36 wherein each different substance in a different well is bound to the surface of the solid support.
38. The array of claim 37 wherein each different substance in a different well is covalently bound to the surface of the solid support.
39. The array of claim 38 wherein each different substance in a different well is covalently bound to the surface of the solid support through a linker.
- 30 40. The array of claim 39 wherein the linker is 3-glycidooxypropyltrimethoxysilane.
41. The array of claim 36 wherein each different substance in a different well is non-covalently bound to the surface of the solid support.
42. The array of claim 36 wherein each different substance in a different well is free of binding to the surface of the solid support.
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43. The array of claim 36 wherein each different substance in a different well is in solution.
44. The array of claim 26 wherein each well contains reagents for assaying biological activity of a protein or molecule.
- 5 45. The array of claim 26 wherein volumes of the wells are between 1 pl and 5  $\mu$ l.
46. The array of claim 26 wherein volumes of the wells are between 1 nl and 1  $\mu$ l.
47. The array of claim 26 wherein volumes of the wells are between 100 nl and 300 nl.
48. The array of claim 26 wherein the bottoms of the wells are square, round, V-shaped or U-shaped.
- 10 49. A method of making a positionally addressable array comprising a plurality of wells on the surface of a solid support comprising the step of:  
casting an array from a microfabricated mold designed to produce a density of wells on a solid surface of greater than 100 wells/cm<sup>2</sup>.
50. A method of making a positionally addressable array comprising a plurality of wells  
15 on the surface of a solid support comprising the steps of:  
(a) casting a secondary mold from a microfabricated mold designed to produce a density of wells on a solid surface of greater than 100 wells/cm<sup>2</sup>; and  
(b) casting at least one array from the secondary mold.
51. The method of claims 49 or 50 wherein the casting of an array further comprises the  
20 steps of:  
(a) covering the mold with a liquid cast material; and  
(b) curing the cast material until the cast is solid.
52. The method of any of claims 49-51 wherein the density of the wells is between 100 and 1,000 wells/cm<sup>2</sup>.
- 25 53. The method of any of claims 49-51 wherein the density of the wells is between 1,000 and 10,000 wells/cm<sup>2</sup>.
54. The method of any of claims 49-51 wherein the density of the wells is between 10,000 and 100,000 wells/cm<sup>2</sup>.
55. The method of any of claims 49-51 wherein the density of the wells is between  
30 100,000 and 1,000,000 wells/cm<sup>2</sup>.
56. The method of any of claims 49-51 wherein the density of the wells is between 1,000,000 and 10,000,000 wells/cm<sup>2</sup>.
57. The method of any of claims 49-51 wherein the density of the wells is between 10,000,000 and 25,000,000 wells/cm<sup>2</sup>.
- 35 58. The method of any of claims 49-51 wherein the density of the wells is greater than 25,000,000 wells/cm<sup>2</sup>.

59. The method of any of claims 49-51 wherein the density of the wells is greater than 10,000,000,000 wells/cm<sup>2</sup>.

60. The method of any of claims 49-51 wherein the density of the wells is greater than 10,000,000,000,000 wells/cm<sup>2</sup>.

61. The method of claim 49 or 50 wherein the array is cast from silicone elastomer.

62. The method of claim 49 or 50 wherein the array is cast from polydimethylsiloxane.

63. The method of claim 51 wherein the liquid cast material is a silicone elastomer.

64. The method of claim 51 wherein the liquid cast material is polydimethylsiloxane.

65. A method of using a positionally addressable array comprising a plurality of different substances, selected from the group consisting of proteins, molecules comprising functional domains of said proteins, whole cells, and protein-containing cellular material, on a solid support, with each different substance being at a different position on the solid support, wherein the plurality of different substances consists of at least 100 different substances per cm<sup>2</sup>, comprising the steps of:

15 (a) contacting a probe with the array; and

(b) detecting protein/probe interaction.

66. A method of using a positionally addressable array comprising a plurality of different proteins, or molecules comprising functional domains of said proteins, on a solid support, with each different protein or molecule being at a different position on the solid support, wherein the plurality of proteins or molecules consists of at least 50% of all expressed proteins with the same type of biological activity in the genome of an organism, comprising the steps of:

20 (a) contacting a probe with the array; and

(b) detecting protein/probe interaction.

67. A method of using a positionally addressable array comprising a plurality of different substances, selected from the group consisting of proteins, molecules comprising functional domains of said proteins, whole cells, and protein-containing cellular material, on a solid support, with each different substance being at a different position on the solid support, wherein the solid support is selected from the group consisting of ceramics, amorphous silicon carbide, castable oxides, polyimides, polymethylmethacrylates, polystyrenes and silicone elastomers, comprising the steps of:

30 (a) contacting a probe with the array; and

(b) detecting protein/probe interaction.

68. A method of using a positionally addressable array comprising a plurality of different substances, selected from the group consisting of proteins, molecules

comprising functional domains of said proteins, whole cells, and protein-containing cellular material, on a solid support, with each different substance being at a different position on the solid support, wherein the plurality of different substances are attached to the solid support via a 3-glycidooxypropyltrimethoxysilane linker, comprising the steps of:

- (a) contacting a probe with the array; and
- (b) detecting protein/probe interaction.

69. The method of any of claims 65-68 wherein the probe is an enzyme substrate or inhibitor.

70. The method of claim 69 wherein the probe is a substrate or inhibitor of an enzyme chosen from the group consisting of kinases, phosphatases, proteases, glycosidases, acetylases, and other group transferring enzymes.

71. The method of any of claims 65-68 wherein the probe is chosen from the group consisting of proteins, oligonucleotides, polynucleotides, DNA, RNA, small molecule substrates, drug candidates, receptors, antigens, steroids, phospholipids, antibodies, glutathione, immunoglobulin domains, maltose, nickel, dihydrotrypsin, and biotin.

72. The method of any of claims 65-68 wherein detection of protein/probe interaction is via mass spectrometry.

73. The method of any of claims 65-68 wherein detection of protein/probe interaction is via a method chosen from the group consisting of chemiluminescence, fluorescence, radiolabeling, and atomic force microscopy.

74. A method of using a positionally addressable array comprising the steps of:

- (a) depositing a plurality of different substances, selected from the group consisting of proteins, molecules comprising functional domains of said proteins, whole cells, and protein-containing cellular material, on a solid support, with each different substance being at a different position on the solid support, wherein the plurality of different substances consists of at least 100 different substances per cm<sup>2</sup>;
- (b) contacting a probe with the array; and
- (c) detecting protein/probe interaction.

75. The method of claim 74 wherein the solid support is a glass slide.

76. A method of using a positionally addressable array comprising the steps of:

- (a) depositing a plurality of different proteins, or molecules comprising functional domains of said proteins, on a solid support, with each different protein or molecule being at a different position on the solid

- support, wherein the plurality of proteins or molecules consists of at least 50% of all expressed proteins with the same type of biological activity in the genome of an organism;
- (b) contacting a probe with the array; and
  - (c) detecting protein/probe interaction.
77. The method of claim 76 wherein the solid support is a glass slide.
78. A method of making a positionally addressable array comprising the steps of:
- (a) casting an array from a microfabricated mold designed to produce a density of wells on a solid surface of greater than 100 wells/cm<sup>2</sup>; and
  - (b) depositing in wells a plurality of different substances, selected from the group consisting of proteins, molecules comprising functional domains of said proteins, whole cells, and protein-containing cellular material, on a solid support, with each different substance being in a different well on the solid support.
79. A method of making a positionally addressable array comprising the steps of:
- (a) casting a secondary mold from a microfabricated mold designed to produce a density of wells on a solid surface of greater than 100 wells/cm<sup>2</sup>;
  - (b) casting at least one array from the secondary mold; and
  - (c) depositing in wells a plurality of different substances, selected from the group consisting of proteins, molecules comprising functional domains of said proteins, whole cells, and protein-containing cellular material, on a solid support, with each different substance being in a different well on the solid support.
80. A method of identifying an antigen that activates a lymphocyte comprising the steps of:
- (a) contacting a positionally addressable array with a plurality of lymphocytes, said array comprising a plurality of potential antigens on a solid support, with each different antigen being at a different position on the solid support, wherein the density of different antigens is at least 100 different antigens per cm<sup>2</sup>; and
  - (b) detecting positions on the solid support where lymphocyte activation occurs.
81. The method of Claim 80 wherein the lymphocytes are derived from a patient.

82. The method of Claim 80 wherein the antigens are selected from the group consisting of antigens of pathogens, antigens of autologous tissues, tissue-specific antigens, disease-specific antigens, and synthetic antigens.
83. The method of Claim 80 wherein lymphocyte activation is detected by measuring antibody synthesis.
84. The method of Claim 80 wherein lymphocyte activation is detected by measuring the incorporation of  $^3\text{H}$ -thymidine by a lymphocyte.
85. The method of Claim 80 wherein lymphocyte activation is detected by determining the expression of cell surface molecules induced or suppressed by lymphocyte activation.
86. The method of Claim 80 wherein lymphocyte activation is detected by determining the expression of secreted molecules induced by lymphocyte activation.
87. The method of Claim 80 wherein lymphocyte activation is detected by measuring the release of  $^{51}\text{Cr}$  chromium.
88. A method of determining the specificity of an antibody preparation comprising the steps of:
- (a) contacting a positionally addressable array with an antibody preparation, said array comprising a plurality of potential antigens on a solid support, with each different antigen being at a different position on the solid support, wherein the density of different antigens is at least 100 different antigens per  $\text{cm}^2$ ; and
  - (b) detecting positions on the solid support where binding by an antibody in said antibody preparation occurs.
89. The method of Claim 88 wherein the antibody preparation comprises antiserum, a monoclonal antibody, or a polyclonal antibody.
90. The method of Claim 88 wherein the antibody preparation comprises Fab fragments, chimeric, single chain, humanized, or synthetic antibodies.
91. The method of Claim 88 wherein antibody binding is detected by contacting the array with a fluorescently labeled secondary antibody that binds to antibody in said antibody preparation; removing unbound secondary antibody; and detecting bound label on the array.
92. A method of identifying a mitogen comprising the steps of:
- (a) contacting a positionally addressable array with a population of cells; said array comprising a plurality of different substances, selected from the group consisting of proteins, molecules comprising functional domains of said proteins, whole cells, and protein-



containing cellular material, on a solid support, with each different substance being at a different position on the solid support, wherein the density of different substances is at least 100 different substances per cm<sup>2</sup>; and

- 5 (b) detecting positions on the solid support where mitogenic activity is induced in a cell.

93. A kit comprising:

- 10 (a) one or more arrays comprising a plurality of wells on the surface of a solid support wherein the density of the wells is at least 100 wells/cm<sup>2</sup>; and  
(b) in one or more containers, one or more probes, reagents, or other molecules.

94. The kit according to Claim 93 wherein said one or more containers comprise a reagent useful for assaying biological activity of a protein.

15 95. The kit according to Claim 93 wherein said one or more containers comprise a reagent useful for assaying interactions between a probe and a protein.

96. The kit according to Claim 94 or 95 wherein the reagent is in solution.

97. The kit according to Claim 94 or 95 wherein the reagent is in solid form.

98. The kit according to Claim 94 or 95 wherein the reagent is contained in each well of the array.

20 99. The kit according to Claim 94 or 95 wherein the reagent is contained in selected wells of the array.

100. The kit according to Claim 93 wherein said one or more containers contain a solution reaction mixture for assaying biological activity of a protein or molecule.

25 101. The kit according to Claim 100 wherein said one or more containers contain one or more substrates to assay said biological activity.

102. A kit comprising:

- 30 (a) one or more positionally addressable arrays comprising a plurality of different substances, selected from the group consisting of proteins, molecules comprising functional domains of said proteins, whole cells, and protein-containing cellular material, on a solid support, with each different substance being at a different position on the solid support, wherein the plurality of different substances consists of at least 100 different substances per cm<sup>2</sup>; and  
35 (b) in one or more containers, one or more probes, reagents, or other molecules.

103. The kit according to Claim 102 wherein the substances are attached to the surface of wells on the solid support.
104. The kit according to Claim 103 wherein the substances are proteins, and the proteins are at least 50% of all expressed proteins with the same type of biological activity in an organism.
105. The kit according to Claim 104 wherein the substances are proteins or molecules comprising functional domains of said proteins, and the proteins or molecules are selected from the group consisting of kinases, phosphatases, proteases, glycosidases, acetylases, nucleic acid binding proteins, and hormone binding proteins.

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